STUDIES ON THE LIFE HISTORY OF SPELOTREMA NICOLLI (TREMATODA: MICROPHALLIDAE) WITH THE DESCRIPTION OF A NEW MICROPHALLID CERCARIA

R. M. CABLE AND A. V. HUNNINEN

(From Purdue University, Oklahoma City University and the Marine Biological Laboratory)

Introduction and Historical Review

Although numerous investigators have observed and correlated certain stages in the life histories of species of *Spelotrema* and related genera of digenetic trematodes, no study has been reported in which the cycle has been completed and proved experimentally. During the summer of 1938, the writers fed metacercariae from the blue crab, *Callinectes sapidus,* to young herring gulls and obtained mature adult trematodes which proved to be a new species, *Spelotrema nicolli* Cable and Hunninen, 1938. At that time, the cercaria could not be found, but during the summer of 1939, the study has been completed and the life history of *S. nicolli* is now well understood.

In 1865, M'Intosh observed encysted microphallid larvae in the tissues of the green crab, *Carcinus maenas.* Lebour (1911) regarded this larva as the metacercaria of *Spelotrema excellens* Nicoll, 1907, and made the significant observation that a stylet was present and the ventral sucker was undeveloped in young metacercariae. Furthermore, she observed that the small monostome xiphidiocercaria, *C. ubiquita* Lebour, 1907, penetrated and encysted in crabs, and postulated that it was the cercaria of *S. excellens.* Brandes (1888) suggested that the metacercaria described by M'Intosh was the larva of *S. claviforme* (Brandes) while Nicoll (1907) maintained that there was a closer agreement between *S. claviforme* and the metacercaria which Lebour (1905) described and later (1911) named *C. littorinae-rudis.* Other microphallid metacercariae that have been found in crustaceans include a form which Villot (1879) found encysted in *Anthura gracilis* and postulated to be the larva of *Levinseniella brachysomum* (Creplin); *C. carcini* Lebour, 1908, from *Corophium grossipes* and *Gammarus duebeni; C. balani* Lebour, 1908, from *Balanus balanoides; C. ligiae* Lebour, 1914, from *Ligia oceanica;* metacercariae of *Levinseniella squatarolae* and *Spelophal-*

lus primas, described by Yamaguti (1934) from *Macrophthalmus dilatatus;* and unnamed *Spelotrema* metacercariae described by Stunkard (1932) from *Porcellanus longicornis* and *C. maenas.*

The observations of Lebour (1911) indicated that cercariae of the *Ubiquita* type may be larval microphallids. Other cercariae of this type include *C. indica LII* and *C. indica LXI* described by Sewell (1922) from *Amnicola travancorica* and *Digoniostoma ceramepoma* respectively; *C. ubiquitoides* Stunkard, 1932, from *Littorina rudis* and *L. littorea;* a similar if not identical form described by Rees (1936) from the same hosts and also *L. obtusata;* and *C. nassicola* Cable and Hunninen, 1938, which occurs in *Nassa obsoleta* and is described more completely in the present paper.

Apparently, certain microphallid cercariae do not leave the molluscan host but lose their tails and encyst in the sporocyst. This behavior seems to be characteristic of *C. crispata* Pelseneer, 1906, in *Natica alderi; C. oocysta* and *C. pirum* found in *Paludestrina stagnalis* by Lebour (1907); *C. sinuosa* Sinitsin, 1911, from *Rissoa venusta; C. dimorpha* Sinitsin, 1911, from *Cerithiolum exille;* and *C. A.* Rothschild, 1936, from *Peringia ulvae.*

Experimental studies have demonstrated portions of the life histories of certain microphallid trematodes. Rothschild (1937) gave an adequate discussion of these studies and reported that she obtained a species of *Maritrema* when encysted *C. oocysta* were fed to gulls. More recently, Sheldon (1938) has found that metacercariae occurring in crayfishes develop into adults of *Maritrema medium* when fed to laboratory mice. Young (1938) has obtained stages in the life cycle of *Levinseniella cruzi* (?) Travassos, the cercaria of which occurs in *Olivella biplicata* and encysts in the sand crab, *Emerita analoga.* Young did not feed metacercariae to birds but concluded from morphological comparisons that the adult was a species occurring in shore birds.

The potential danger of species of *Spelotrema* as human parasites has been demonstrated by Africa and Garcia (1935), who found *Heterophyes brevicaeca* (renamed *Spelotrema brevicaeca* by Tubangui and Africa, 1938) associated with acute cardiac dilatation and discovered the eggs of this parasite in the heart tissue.

Materials and Methods

Throughout the present investigation, living material has been studied as much as possible since it was found to be far more favorable than fixed and stained specimens for most observations. Since all crabs contained metacercariae from natural infections, it was necessary to isolate

those for experimental purposes for a time sufficient to permit encystment and growth of any recently acquired parasites that might have been present. In this manner, experimental infections could be detected by finding migrating larvae or very small metacercariae.

In studying the larger metacercariae, considerable difficulty was experienced in removing the larvae from the cysts without injury. This was done most successfully with the aid of a small glass tube drawn out and cut off with scissors at a point where the diameter was slightly smaller than that of the cyst. With aid of forceps, it was possible to rupture the cyst by forcing the capillary glass tube over it. Birds were infected by feeding them several hundred metacercariae placed in the body cavity of small fishes. Development of the eggs was followed by teasing adult worms and keeping the eggs thus obtained for study in dishes of sea water which was changed twice daily.

Conventional methods were employed in studying the various stages, neutral red and Nile blue sulphate being used for *intravitam* staining, paracarmine for whole mounts, and Delafield's haematoxylin with eosin counterstain for sectioned material. Unstained metacercariae in the crab's tissues were dehydrated and mounted in damar and also passed gradually into glycerine and mounted in glycerine jelly. All drawings except those indicated as diagrammatic were made with the aid of projection equipment, some of the details being added free-hand. All measurements given below are in millimeters and are from living material except in the case of adults which were stained whole mounts.

Observations

The Experimental Proof of the Life History

It has been known for several years that practically every blue crab in the Woods Hole area is infected with a microphallid metacercaria. Before attempting to obtain the cercarial and adult stages, a careful study of natural infections was made to find out as much as possible concerning the nature of the cercaria and the adult. Since the older metacercaria was well differentiated, it could be identified positively as a species of *Spelotrema*. During the summer of 1938, each of three young herring gulls was given an initial feeding of metacercariae, a second feeding 15 days later, and killed after three days. All three birds yielded large numbers of adult worms which could be separated readily into two size groups corresponding to the ages of the infections. Three control birds were negative for the same species but contained other parasites as did the experimental animals. In examining the birds, the cloaca and large intestine were severed just above the ceca and the small intestine divided into approximately four-inch lengths which were examined separately.

Most of the worms occurred in the two segments of the small intestine above the terminal one although a few were recovered both above and below this level. They were never found in the ceca. During the summer of 1939, three additional birds were infected experimentally, one being fed metacercariae twice, 17 and 3 days before it was killed and examined, another twice, 25 days and 12 hours, while the third was killed 36 hours after a single feeding of cysts. Since all of these birds became heavily infected and three additional controls were negative, it is concluded that the worms recovered were adults of the metacercariae fed. This conclusion is supported by the close agreement between the older metacercaria (Fig. 7) and the adult (Fig. 10).

A careful study of young metacercariae gave information of considerable significance in the search for the cercaria. It was determined that the cercaria would be of the Ubiquita type since a large stylet was present and the ventral sucker was undeveloped, From very young metacercariae, the exact size and shape of the stylet were determined.

During the summer of 1938, we were able to find only one cercaria of the Ubiquita type and this occurred in *Nassa obsoleta* collected at Sippewissett. Three small blue crabs were exposed to large numbers of these cercariae with negative results. This was expected since there were considerable differences in the shape and size of the stylets of young metacercariae and the cercariae from *N. obsoleta.* An examination of several thousand *N. obsoleta,* collected at Waquoit Bay where infected crabs were abundant, did not yield a single infection with the species found at Sippewissett. In view of these results, it was concluded that the cercaria of *S. nicolli* remained to be found. Early in the summer of 1939, the study was resumed from an ecological viewpoint. Due to the migratory habits of the blue crab, it was necessary to determine first whether infection occurred in open water or after migration into bays and inlets. To answer this question, crabs were collected from Waquoit Bay at Menauhant which is at least four miles by water from the only entrance to the bay. Since these crabs contained very young metacercariae only 0.05 mm. in diameter, it seemed probable that the infections were acquired in the bay. Then followed a systematic collection and examination of all species of mollusks that could be found where infected crabs occurred. Large numbers of *Nassa obsoleta, N. vibex, Littorina littorea, L. rudis, Melampus bidentatus, Mitrella lunata, Crepidula fornicata, Venus mercenaria, Modiolus modiolus,* and *Pecten irradians* were examined and found negative for ubiquitous cercariae. Finally, a larva of the type sought was found in the very small snail, *Bittium alternatum,* which occurred in large numbers on seaweed grow-

ing in one to four feet of water. The cercaria seemed to be identical with the form in the blue crab, especially in respect to the size and shape of the stylet. To test this apparent relationship, a small crab that had been isolated for three weeks was exposed to the cercariae almost continuously for seven days before it was killed. Upon examination, certain fibers were found to contain not only a few older cysts from natural infections but also numerous very young metacercariae (Fig. 19) most of which had not encysted. The young encysted larvae were undoubtedly from the experimental infection and identical with those recovered repeatedly from naturally infected crabs that had been collected only a short time before examination. Two additional crabs were exposed to cercariae and both became heavily infected.

The manner in which the cercariae entered the crab remained to be determined. The larva is not a powerful swimmer and when disturbed it usually ceases swimming for a few seconds. This behavior suggested that when the larvae come near the crab and are disturbed by its respiratory activity, they are carried passively into the gill chamber. With this possibility in mind, a small crab was exposed for 45 minutes to a large number of cercariae and then killed and examined. Two active larvae were found in the heart and a number was recovered from the efferent branchial vein of each gill. Since no larvae were recovered from the afferent vessels, it seems that they either penetrated the gill lamellae and passed by way of the outer lamellar sinuses to the efferent vessels, or bored directly into the efferent veins. The fact that no larvae were observed in the lamellae indicates that the cercariae normally lodge in the interlamellar spaces, which afford excellent protection, and make their way to the bases of the lamellae where they bore directly into the efferent veins (Fig. 18).

Description of Stages in the Life History of Spelotrema nicolli

Adult (Figs. 9–12).

Specific Diagnosis.—Microphallidae with characters of the genus *Spelotrema*. Total length 0.51–.58 (average 0.54); maximum width of forebody 0.21–.27 (0.24); width at mid-body 0.21–.25 (0.23); width hind-body 0.32–.37 (0.34); width oral sucker 0.05–.06 (0.056), ventral sucker 0.05–.065 (0.058). Distance from center of ventral sucker to posterior end of body 0.2–.24 (0.22). Maximum length prepharynx 0.035; esophagus length 0.12–.2 (0.16); ceca average 0.14 long, divergent, and not reaching posterior margin of ventral sucker. Diameter of penis 0.019–.024 (0.021); seminal vesicle 0.07–.09 (0.085) by 0.015–.045 (0.035). Testes about equal in size, 0.1–.13 (0.11) by 0.05–.075 (0.06). Ovary right, anterior and adjacent to testis of that side, 0.08–.09 (0.085) by an average of 0.05. Laurer's Canal present, shell gland dorsal to oötype; uterus voluminous, extending anterior to testes as far as ceca. Vitellaria diffuse, not observed as distinct lobes. Eggs very numerous, 0.018–.022 long by 0.009–.011 wide.

Host (experimental). *Larus argentatus,* localizing near posterior end of small intestine.

Locality. Woods Hole, Massachusetts, U. S. A.

Type specimens. Holotype No. 9232, Helminthological Collection, U. S. National Museum; paratypes in authors' collections.

The forebody of *S. nicolli* is armed with prominent scale-like spines arranged in imbricated rows. These spines decrease in number and size at the level of the acetabulum but may be found by careful examination to be scattered in small numbers over the hind-body, almost to the posterior end. Prominent refractile glands are scattered beneath the cuticula of the forebody and are so numerous that other structures in that part of the body are obscured in living specimens. A cluster of similar glands have ducts which converge at the margin of the genital pore (Fig. 11) and might be mistaken for the deeper prostate glands. The cytoplasm of the subcuticular glands is homogeneous in appearance. The ceca have thick walls of gland-like cells and are surrounded by prominent masses of granular cells having the appearance of glands.

The excretory system of the adult is the same as described below for the older metacercaria. The reproductive system as a whole may be observed much easier in living worms than in either whole mounts or sectioned material. Since the presence of numerous eggs in the uterus obscured many details of the reproductive system, some of the feeding experiments were devised primarily to provide young worms in which only a few eggs were present. The details of structure and the functioning of various generative organs were observed very favorably when such worms were mounted dorsal side up under as little coverglass pressure as possible and studied with the oil immersion objective.

The penis (Figs. 9–12) is a pyriform, muscular organ, situated in the genital atrium at the left of the ventral sucker. It is penetrated by the ejaculatory duct which seems to receive the openings of a number of elongate prostate glands just before it enters the penis. The ejaculatory duct is moderately sinuous. It expands to form the seminal vesicle, there being no indication of a double-walled vesicle. On the right, the vesicle narrows to form the short vas deferens which extends posteriorly, dorsal to the ventral sucker, and divides to form the delicate vasa efferentia. From this Y-shaped division, the vasa efferentia pass posterolaterally, very close to the dorsal side of the body, and connect with the testes.

The ovary is frequently somewhat triangular in shape. From the median edge, the oviduct extends posteriorly as a thick-walled, sinuous tube. Close to the median edge of the right testis, the oviduct enters a prominent, spherical bulb which has thick, muscular walls. Because of its function as described below, this bulb is called the ovijector. A small

internal papilla marks the point at which the oviduct enters the ovijector, and a more prominent papilla at the opposite side of the bulb surrounds the entrance to the oötype (Fig. 12). Proceeding from the ovijector, the oötype turns abruptly ventrad and is joined immediately by the Laurer's Canal which extends dorsally in a sinuous path and opens near the median line. From the ovijector to the opening of Laurer's Canal, the wall of the oötype is composed of overlapping cells. Beyond the opening, the wall is thinner. The oötype extends posteriorly for a short distance and then bends dorsally, extending anteriorly not far beneath the dorsal surface of the body. It is ciliated almost to the point where the dorsal loop receives the opening of the vitelline reservoir. Just anterior to this opening, the oötype receives the numerous slender ducts of the shell gland, all of which lies above the oötype. The vitellaria are composed of diffuse masses situated below and behind the testes. The vitelline ducts extend forward below the testes and unite in the median line to form the vitelline reservoir which passes backward and upward to join the oötype.

When living worms are studied as has been described, the process of egg formation can be observed in considerable detail. The oöcyte (assuming that meiotic divisions do not occur until sperm penetration, as is true of certain other trematodes) gradually separates from the ovarian mass while the preceding egg is formed. It is started and propelled down the oviduct, however, by a constriction passing along the oviduct. The oöcyte moves slowly at first and then with increasing rapidity. It remains for only an instant in the ovijector which contracts immediately, expelling the oöcyte forcibly into the oötype. This contraction of the ovijector is followed by a localized enlargement of the oötype in which currents produced by cilia rotate the oöcyte rapidly for a few moments. Then the oöcyte is carried along the oötype by a series of contractions. Since a few sperms are usually present in the ovijector, fertilization (i.e., sperm penetration) could occur there. Sperms were seen attempting to pass up the oviduct as the oöcyte descended but have never been observed in the ovary or oviduct during its inactive phase. The passage between the ovijector and the oviduct remains closed most of the time. Since the ovijector does not always contain sperms and the oöcyte passes through it almost too rapidly to be observed, it seems probable that fertilization occurs normally in the oötype which is always crowded with sperms. The rapid and fairly prolonged rotation of the oöcyte in the oötype would facilitate fertilization by aiding the sperm to engage and coil about the oöcyte.

The zygote pauses at the opening of the vitelline reservoir which

then begins to contract. These movements become more and more forcible until several masses of vitelline material are expelled into the oötype. The zygote, surrounded by this material, then passes just beyond the openings of the shell gland into the more muscular part of the oötype which may be termed the egg chamber. Excess vitelline masses and occasional sperms are swept by the cilia of the oötype back to the Laurer's Canal through which they escape from the body. Before the shell is formed, vigorous contractions of the egg chamber break up the masses of vitelline material into fine granules. The egg is kept in constant motion by contractions of the oötype and may be turned end for end several times before the shell is formed completely. A much stronger contraction begins back of the completed egg and ejects it into the uterus. Under observation, several attempts may be necessary to expel the egg, due perhaps to the interference of coverglass pressure with normal function. At the moment the egg passes into the uterus another oöcyte begins to move down the oviduct and the process is repeated. In one instance, it was noted that at room temperature 24 minutes elapsed between the descent of one oöcyte and that of the succeeding one. At the body temperature of the host, however, egg formation must proceed much more rapidly than observed to account for the number of eggs present in young worms.

The eggs are colorless when first formed but become yellow with age. Small, abnormally shaped eggs (Fig. 13) with terminal knobs are often seen in the uterus and such eggs are produced frequently after removal of the worms from the host. Occasionally, a specimen is observed in which practically all the eggs are of this type. Abnormal eggs almost always contain only vitelline material and are formed apparently when oöcytes fail to descend.

The more significant measurements of described species of *Spelotrema* are given in Table I. As Odhner (1905) pointed out, the size of the penis is remarkably constant for a given species and this character alone distinguishes *S. nicolli* from all other species of *Spelotrema* except *S. pygmaeum* (Levinsen). However, *S. nicolli* and *S. pygmaeum* differ significantly in respect to body size and shape, sucker ratios, extent of the uterus, and nature of the vitellaria. Both Odhner and Nicoll (1909) gave 0.5 mm. as the maximum length of *S. pygmaeum;* practically every stained specimen of *S. nicolli* exceeds this length and the average for moderately contracted living specimens is over 0.6 mm. Concerning the body shape of *S. pygmaeum,* Odhner stated that, at all degrees of contraction, the width of the body increased without interruption from the

anterior towards the broadly rounded posterior end. He observed this body form in all of his material and considered it a specific character in differentiating *S. pygmaeum* from *S. simile* (Jägerskiöld, 1900). Except when extended greatly, the body of *S. nicolli* is constricted at the

TABLE I

Comparison of species of Spelotrema

Species	*S. pygmaeum*	*S. claviforme*	*S. simile*	*S. excellens*	*S. brevicaeca*	*S. nicolli*
	(Levinsen)	(Brandes)	(Jägerskiöld)	Nicoll	(Africa and Garcia)	Cable and Hunninen
Citation	Odhner (1905)	*Nicoll (1907)	Odhner (1905)	*Nicoll (1907)	Tubangui and Africa (1938)	Present Report
Length (mm.)	Not exceeding 0.5	0.23–.4	0.45–.6	0.71–1.39 (av. 0.91)	0.5–.7	0.51–.58 (av. 0.54)
Breadth (maximum) (mm.)	0.2–.3	0.17		0.37–.49 (av. 0.41)	0.3–.4	0.32–.37 (av. 0.34)
Oral sucker diameter (mm.)	0.04–.053	0.038	0.046–.058	0.068–.086 (av. 0.076)	0.065–.095	0.05–.06 (av. 0.056)
Ventral sucker diameter (mm.)	0.037–.048	0.031	0.049–.062	0.062–.081 (av. 0.071)	0.08–.105	0.05–.065 (av. 0.058)
Male papilla diameter (mm.)	0.021–.023	0.013–.014	0.04	0.05–.065	0.03–.045	0.019–.024 (av. 0.021)
Eggs	0.02–.023 by 0.012	0.02–.024 by 0.011–.014	0.023–.026	0.023–.025 by 0.01–.013	0.015–.016 by 0.0094–.01	0.018–.022 by 0.009–.011

* An obvious misplacement of the decimal point in several of Nicoll's measurements has been corrected.

level of the ventral sucker. This characteristic is noticed particularly in living specimens and, although present in stained material, may be rendered less prominent by pressure when specimens are fixed under the coverglass. According to Odhner, the oral sucker of *S. pygmaeum* is usually larger than the ventral sucker, although exceptions were noted. The opposite is true of *S. nicolli;* the ventral sucker is nearer the middle of the body than in *S. pygmaeum.* In *S. nicolli,* the hind-body is larger and more expanded than in *S. pygmaeum.* In Odhner's figure, the uterus of *S. pygmaeum* does not extend anterior to the testes and this condition may be regarded as typical since he had an abundance of material. In *S. nicolli,* the uterus always extends anterior to the testes; this condition is characteristic even of the empty uterine coils of immature worms, especially on the antovarian side, and does not depend

on the degree of maturity. Odhner's figure also shows approximately 100 eggs in the uterus of *S. pygmaeum*. This number is exceeded in specimens of *S. nicolli* that have been in the bird only 36 hours and the uterus of older worms contains several hundred eggs. Odhner also described eight distinct vitelline masses on each side of *S. pygmaeum* and represented them as a cluster somewhat posterior to the testes. In *S. nicolli,* the vitellaria are more diffuse and overlap the posterior edge of the testes. In some specimens, vitelline lobes have been observed but they are fused in such a manner that a count can not be made with certainty. A further difference between *S. nicolli* and *S. pygmaeum* is indicated by Nicoll's (1909) statement that the ceca of *S. pygmaeum* reach the posterior margin of the ventral sucker; the ceca of *S. nicolli* rarely extend posterior to the middle of the sucker.

The metacercaria (Figs. 4–8).

The metacercariae occur only in certain slender fibers which extend from the viscera of the crab to the bases of the legs. These fibers are very tough and elastic and seem to be composed of connective tissue since they are not striated and separate readily from the groups of striated muscles at the bases of the legs. In cases of heavy infection, the fibers are greatly enlarged and filled with metacercariae.

Metacercariae were observed ranging from 0.05 to almost 0.5 mm. in cyst diameter. The stylet is retained until the metacercaria attains considerable size. It is gradually absorbed, however, and never shed or broken off as in the case of some xiphidiocercariae. The young metacercaria secretes a very thin primary cyst wall (Fig. 5*a*) which is elastic and changes shape with the movements of the worm. At a later stage, the cyst becomes enclosed in a mass of granular tissue which is of host origin and contributes to the formation of secondary cyst layers. In the older metacercaria (Fig. 8), the cyst membranes are of two types, an outer radially striated layer which is very thick and exceedingly tough, and one to three hyaline inner layers which become fairly thick. The entire cyst may be embedded in a mass of fibrous tissue of host origin.

When removed from the cyst, the older metacercaria is practically as large as the adult which it resembles closely except that eggs are absent. After excystation, egg production must begin in a short time since the young adults contain eggs after only 12 hours in the avian host.

The excretory formula of the metacercaria (Fig. 6) is $2[(2+2)+(2+2)]$; it remains unchanged in the adult stage.

Cercaria (Figs. 16, 17).

Specific Diagnosis.—Small "monostome" xiphidiocercaria with the characters of the Ubiquita Group of Sewell (1922). Body contracted less than 0.1 long, extended 0.24, average 0.11; covered with small scale-like spines in imbricated rows. Tail 0.04–.1 long, with fine cuticular annulations. Oral sucker 0.026 long, provided with stylet 0.016 long. Remainder of digestive system not observed. Four pairs of cephalic glands of two types, a larger anterior pair on each side with ducts extending forward together and then separating, the median duct crossing the oral sucker and joining the lateral duct at the side of the sucker where both turn ventrad, opening on ventral surface of body near anterior end; a smaller posterior pair with ducts passing anteriorly together with lateral duct of anterior gland and opening near tip of stylet. Excretory vesicle U-shaped, each arm receiving a main collecting tubule which extends forward half way to cephalic glands, there dividing to form anterior and posterior secondary tubules, each of which receives capillaries of two flame cells. Excretory formula: $2[(1+1)+(1+1)]$. Develop in oval or elongate sporocysts with or without terminal knobs.

Host: *Bittium alternatum* (Say).

Locality: Woods Hole, Massachusetts, U. S. A.

The cercaria of *S. nicolli* resembles other larvae of the Ubiquita type but differs from all described species in the nature of its penetration glands, size and shape of stylet, and molluscan host. The stylet is symmetrical in dorsal aspect but asymmetrical when viewed from the side. The barb is quite hard and persists whereas the shaft is fragile and frequently disintegrates when living cercariae are mounted under consider-

---

Explanation of Plate I

(Figs. 1–3, *Cercaria nassicola*)

Fig. 1. Cercaria in dorsal aspect.

Fig. 2. Stylet, ventral aspect.

Fig. 3. Anterior end of cercaria in side view, showing arrangement of cephalic gland ducts.

(Figs. 4–6, *Spelotrema nicolli*)

Fig. 4. Older metacercariae in tissue of crab.

Fig. 5. *A.* Younger metacercaria in which stylet is still present. *B.* Older metacercaria.

Fig. 6. Excretory system of metacercaria.

Abbreviations

AO, anterior openings of posterior cephalic glands.
DG_1, anterior cephalic gland duct.
DG_2, posterior cephalic gland duct.
DO, dorsal opening of anterior cephalic gland duct.
G_1, anterior cephalic glands.
G_2, posterior cephalic glands.
S, stylet.
VO, ventral opening of anterior cephalic gland duct.

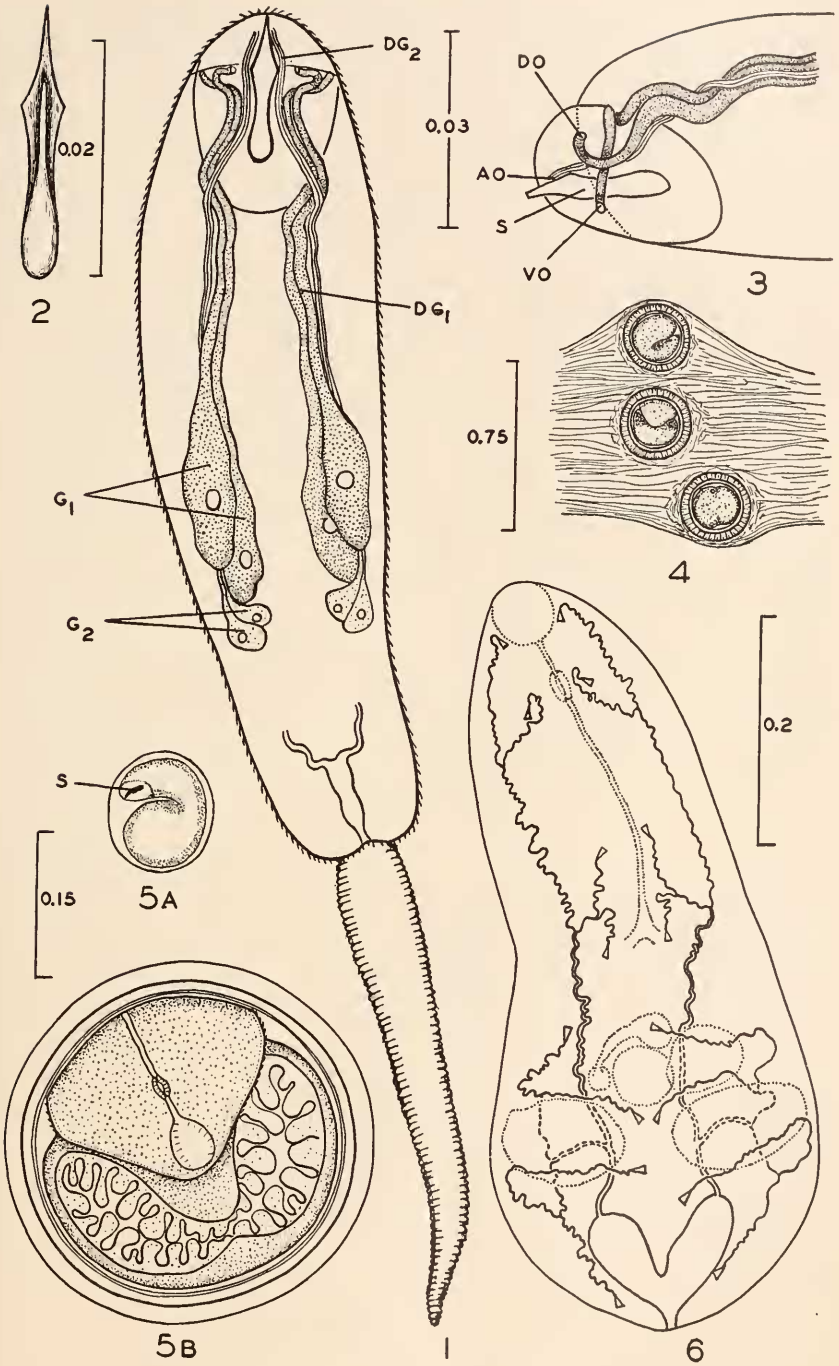

PLATE I

able coverglass pressure. On each side, the anterior pair of cephalic glands stains intensely with neutral red and Nile blue sulphate. Their contents move readily into the ducts which consequently become very conspicuous. The two posterior glands do not stain appreciably with *intra-vitam* stains and their ducts are extremely difficult to observe in cercariae emerging spontaneously from the snail. However, the cercariae encyst readily on the slide and then the ducts of the posterior glands become distinct. Before such encystment, the contents of the anterior glands are thrown out and their ducts become invisible. The ducts of both types of glands have been observed simultaneously only a few times, usually in larvae obtained by crushing the snail. Encystment on the slide occurs very rapidly, especially when *intra-vitam* stains are used. Cystogenous material is secreted over the entire surface of the body, the tail is lost, and the cyst forms with the body more or less extended instead of flexed as in normal encystment in the crab. The larva sometimes ruptures the cyst and emerges but dies after a short time.

Upon emerging from the snail, the cercariae swim almost continuously with the posterior part of the body flexed ventrally and the tail lashing vigorously. During short rest periods, the body remains flexed for a moment and then usually extends and contracts once or twice before swimming is resumed. It has been mentioned already that swimming may cease in response to stimulation. To observe this behavior more closely, 10 cercariae were isolated in separate culture dishes and each was stimulated five times at 15-second intervals by dipping a needle

---

Explanation of Plate II

(All figures concern *Spelotrema nicolli*.)

Fig. 7. Morphology of metacercaria removed from cyst.

Fig. 8. Details of cyst structure.

Fig. 9. Slightly diagonal cross-section of adult, showing details of the genitalia.

Fig. 10. Adult. Holotype in ventral aspect.

Abbreviations

E, esophagus.
ED, ejaculatory duct.
EV, excretory vesicle.
GA, genital atrium.
GO, opening of subcuticular gland.
GP, genital pore.
I, intestine.
MP, male papilla or penis.
OS, oral sucker.
OV, ovary.
PH, pharynx.
PP, prepharynx.
PR, prostate cells.
SV, seminal vesicle.
T, testis.
U, uterus.
V, vitellaria.
VA, vagina.
VD, vitelline duct.
VS, ventral sucker.

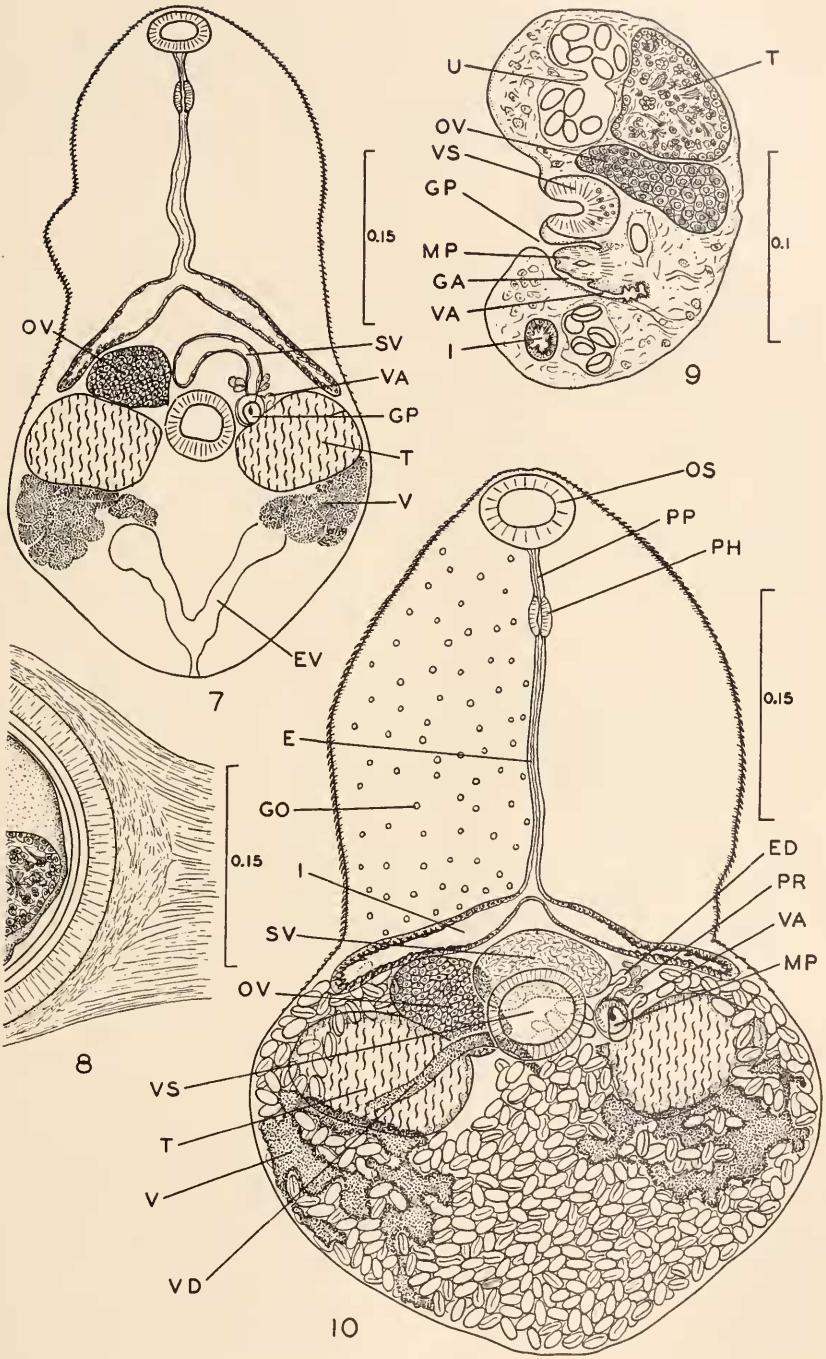
OV
SV
VA
GP
T
V
EV
0.15
7
U
T
OV
VS
GP
MP
GA
VA
I
0.1
9
OS
PP
PH
0.15
E
GO
I
SV
OV
0.15
8
ED
PR
VA
MP
VS
T
V
VD
10

PLATE II

into the water. Four larvae responded to every stimulus with immediate cessation of swimming, three responded to four of the five stimulations, two to three, and one to only a single stimulus. Of a total of fifty observations, 39 gave positive responses, swimming movements ceasing in each case for 1.3 to 7.7 seconds, with an average of 2.9 seconds.

The sporocyst (Fig. 15).

There are probably two sporocyst generations of *S. nicolli* in *B. alternatum* but only that giving rise to cercariae has been observed. The smallest sporocyst observed measured 0.06 mm. long. The younger sporocysts are oval in shape and contain no mature cercariae, while the older ones are elongate and frequently constricted at one or both ends. The birth pore is terminal. It is rather difficult to separate some of the knobbed sporocysts from the host tissue and when they are removed, there is evidence in some cases that parts are broken off. The constricted sporocysts might be interpreted either as indicating fission or

---

Explanation of Plate III

(All figures concern *Spelotrema nicolli.*)

Fig. 11. Adult. Free-hand sketch showing group of subcuticular glands with ducts converging at the genital pore.

Fig. 12. Reproductive system of adult (semi-diagrammatic).

Fig. 13. Normal and abnormal eggs.

Fig. 14. Embryonated egg containing miracidium.

Fig. 15. Sporocysts.

Fig. 16. Cercaria, dorsal view.

Fig. 17. Stylet of cercaria, (*A*) dorsal and (*B*) lateral views.

Fig. 18. Diagram indicating probable route of cercariae in penetrating the crab's gill.

Fig. 19. Portion of a fiber of crab's tissue containing young metacercariae from experimental infection.

Abbreviations

AF, afferent branchial vessel.
BP, birth pore.
DG_1, anterior cephalic gland duct.
DG_2, posterior cephalic gland duct.
ED, ejaculatory duct.
EF, efferent branchial vessel.
EV, excretory vesicle.
G_1, anterior cephalic glands.
G_2, posterior cephalic glands.
GL, gill lamella.
LC, Laurer's Canal.
MP, male papilla or penis.
OD, oviduct.
OJ, ovijector.
OO, oötype.
OS, oral sucker.
OV, ovary.
PR, prostate cells.
S, stylet.
SG, shell gland.
SV, seminal vesicle.
T, testis.
U, uterus.
VA, vagina.
VD, vitelline duct.
VR, vitelline reservoir.
VS, ventral sucker.

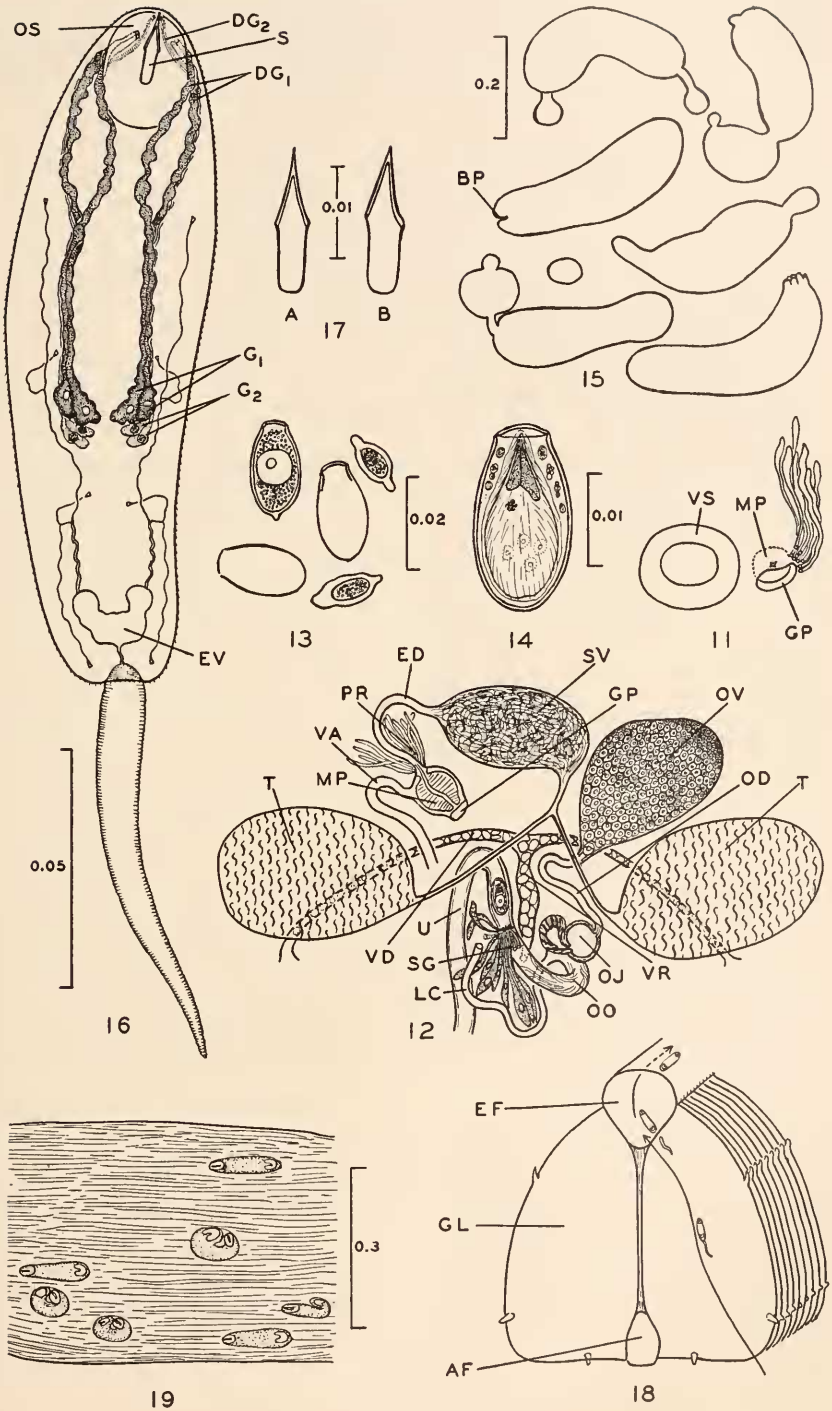
OS
DG2
S
DG1
G1
G2
EV
0.05
16
0.01
A
B
17
0.2
BP
15
0.02
13
0.01
14
VS
MP
GP
11
ED
SV
PR
GP
OV
VA
MP
T
OD
T
U
VD
SG
OJ
VR
LC
OO
12
0.3
19
EF
GL
AF
18

PLATE III

merely as older sporocysts, portions of which are exhausted. Other ubiquitous cercariae develop in oval sporocyts, a fact which affords an additional difference between the cercaria of *S. nicolli* and described species.

The egg and miracidium (Figs. 13, 14).

The process of egg formation has been described above. Development of the miracidium is exceedingly difficult to follow because of its small size. When gravid adult worms were teased, a portion of the eggs obtained developed in a normal manner when placed in sea water which was changed frequently to provide oxygen. Development observed in these eggs seemed to be extremely slow and although it was followed for 30 days before the study had to be terminated, the miracidia showed no signs of activity.

The first part of the micracidium to appear distinctly is the anterior end which is seen as a small, granular cone directed toward the operculum. As development proceeds, the remainder of the miracidium becomes more definite in outline. The granules filling the anterior end appear to be the contents of gland cells similar to those of other miracidia. Just posterior to this granular mass is a spherical cluster of granules which may be an eye-spot. Except for a few germ cells, the remainder of the miracidium is poorly defined. It is quite possible that the miracidia died before development was completed since movement was never observed.

Description of Cercaria nassicola Cable and Hunninen, 1938 (*Figs. 1–3*)

Specific Diagnosis—Small "monostome" xiphidiocercaria with the characters of the Ubiquita group. When moderately contracted, body length 0.1–.13, tail 0.07–.08. Entire body covered with prominent retrorse spines; tail finely annulated. Oral sucker 0.03 long; stylet 0.023 in length with tip truncate in lateral aspect. Two pairs of cephalic glands on each side, the anterior pair larger and with coarse granules, the posterior pair smaller and with finer granules. Larger glands stain intensely with neutral red, with one duct opening dorsolaterally, the other ventrolaterally in a groove encircling anterior end of body; smaller glands stain less intensely with neutral red, ducts open near tip of stylet. Develop in small oval sporocyts.

Host *Nassa obsoleta* (Say).

Locality: Woods Hole, Massachusetts, U. S. A.

C. nassicola is very delicate and disintegrates in a short time when subjected to any considerable coverglass pressure. For this reason, it has not been possible to determine the excretory formula. The vesicle and the beginning of the main collecting tubules were observed clearly only a few times. Decaudation occurs in a short time when the larvae are mounted for study but encystment has not been observed.

In a preliminary description of *C. nassicola,* it was stated that the tail is spinose. Further study has proved that this is not the case, but instead, the tail is very similar to that of the cercaria of *S. nicolli.* When the living cercariae are mounted for study, the cuticle of the tail becomes detached at the posterior margin of each annulation as disintegration begins and gives the tail a spinose appearance. This condition was encountered so consistently in earlier observations that it was misinterpreted.

The size and shape of the stylet and the arrangement of the cephalic gland ducts of *C. nassicola* are characteristic of the species. The ducts of the larger glands extend forward in the dorsal part of the body, a pair on either side. At about the middle of the oral sucker, the median duct of each pair loops upward, opening dorsolaterally in the groove. There is no marked separation of the ducts anteriorly as in the cercaria of *S. nicolli* and *C. ubiquitoides.* The ducts of the smaller posterior glands are very delicate. They extend anteriorly at the sides of the larger ducts which they cross at the posterior end of the oral sucker. From this point, they turn downward and forward around the oral sucker to open near the tip of the stylet.

Discussion

There is some confusion concerning the zoölogical status of the genera *Spelotrema* and *Levinseniella.* Tubangui and Africa (1938) expressed hesitation in referring *Heterophyes brevicaeca* to the genus *Spelotrema.* Young (1938) did not figure the adult stage of the species he referred to the genus *Levinseniella* and his incomplete description of this stage could have been that of a species of either *Levinseniella* or *Spelotrema.* As a matter of fact, the difference between these genera is quite evident but the manner in which the present conception of them has developed is confusing.

Stossich (1899) erected the genus *Levinsenia* to include all the microphallids known at that time, viz., *Distomum opacum* Ward, *D. brachysomum* Creplin, *D. pygmaeum* Levinsen and *D. macrophallos* von Linstow. In the same year, Lühe and Looss independently designated *L. brachysoma* as type species. Ward (1901) removed *L. opacum* to the new genus *Microphallus* and stated that Stiles and Hassall were to propose the name *Levinseniella* to replace *Levinsenia* (preoccupied) in a forthcoming paper which did not appear until 1902. Meanwhile, Jägerskiöld (1901), with full knowledge of Ward's statement concerning the intention of Stiles and Hassall, proposed the name *Spelotrema* for *Levinsenia* and designated *S. pygmaea* as type. If the law of priority

should be applied to this case, *Spelotrema* Jägerskiöld, 1901, should be suppressed as a synonym of *Levinseniella* Stiles and Hassall in Ward, 1901, since Jägerskiöld stated subsequently (1904) "Spelotrema (= Levinseniella)" and therefore certainly regarded them as synonymous. His later (1907) conception of two distinct genera is valid, however, and must be accepted although he should not have retained for them names which he had regarded previously as synonyms. To suppress *Spelotrema* as a synonym of *Levinseniella,* and propose a new generic name for the species at present allocated to the genus *Spelotrema,* would probably increase rather than diminish the present confusion. For this reason, the writers are inclined to let the matter stand.

As regarded at present, members of the genus *Spelotrema* have a simple genital atrium containing a conical male papilla or penis, near the base of which the vagina enters the atrium. Species of *Levinseniella* have very different genitalia with complicated folding of the genital atrium. These generic differences are discussed fully by Jägerskiöld (1907).

Much emphasis has been placed in recent years on the value of information obtained from life history studies and excretory patterns in determining the relationships of digenetic trematodes. This information is gradually providing a natural system of classification based on fundamental relationships. As a result, it has been discovered that the taxonomic importance of certain morphological characters has been overemphasized in the past, particularly in the separation of familial and more inclusive groups. Studies on life histories and excretory systems have revealed in some instances hitherto unexpected relationships between morphologically dissimilar adult trematodes. On the other hand, it has been discovered that fundamentally dissimilar groups have been regarded as closely related by earlier workers who were misled by apparent morphological resemblances.

Perhaps no group of trematodes illustrates these facts more clearly than those that were included in the family Heterophyidae until only a few years ago. On the basis of morphological studies, Witenberg (1929) excluded certain genera from this family and proposed to unite those remaining with the opisthorchiids in the superfamily Opisthorchoidea. The life history studies of Stunkard (1930) on *Cryptocotyle lingua* and Vogel (1934) on *Opisthorchis felineus* gave such convincing evidence of the close affinities of the Heterophyidae and Opisthorchiidae that Vogel concurred in the opinion of Witenberg, renaming the superfamily Opisthorchioidea. Previously, Travassos and Viana had raised the heterophyid subfamily, Microphallinae Ward, to the status of a family, Microphallidae, to include *Microphallus* and related genera,

all of which were among those Witenberg removed from the Heterophyidae. The observations of Lebour (1911) gave the first significant indications as to the nature of microphallid life histories. Subsequent studies afford indisputable evidence of the validity of placing *Spelotrema* and related genera in a distinct family. The validity of the name of this family, Microphallidae, depends on a more complete understanding of the morphology and life history of *Microphallus,* the type genus. However, the exact agreement between the excretory systems of *Microphallus, Spelotrema,* and *Maritrema* and other morphological similarities make it seem very likely that the retention of the name *Microphallidae* will be justified by further studies.

Rothschild (1937) and Stunkard (1938) suggested that the microphallids may be related to the Lecithodendriidae, a family which McMullen (1937) has placed in the superfamily Plagiorchoidea. On the basis of Carrère's (1936) study of the life history of *Maritrema rhodanicum,* McMullen places *Maritrema* in the Lecithodendriidae since Carrère reported that the cercaria of *M. rhodanicum* is of the Armatae type. In the writers' opinion, families of the Plagiorchoidea differ as significantly in respect to morphology and life history as do certain plagiorchoids and microphallids. It therefore seems reasonable to include the Microphallidae in the Plagiorchoidea.

The terminal portions of the reproductive system seem to be subject to extreme modifications as indicated by the differences observed in the microphallid genera, *Spelotrema, Maritrema, Levinseniella,* and *Microphallus.* The Heterophyidae (*sensu stricto*) also show considerable variation in the genitalia and genital suckers are by no means limited to this group. It is clear, then, that the apparent nature of the reproductive system, particularly the terminal portions, has been misleading in the separation of suprageneric groups, especially when careful studies of the genitalia have not been correlated with other characteristics.

Summary

The life history of *Spelotrema nicolli* Cable and Hunninen, 1938, has been traced experimentally and the various stages have been described. The cercaria develops in sporocysts in the digestive gland of *Bittium alternatum* (Say). The blue crab, *Callinectes sapidus* Rathbun, serves as the second intermediate host. In experimental infections, the cercariae were found to penetrate the gills, and pass by way of the blood stream to the tissues. Metacercariae from naturally infected crabs were fed to young herring gulls, *Larus argentatus* Pontoppidan, from all of which numerous adult *S. nicolli* were recovered.

A re-description of *Cercaria nassicola* Cable and Hunninen, 1938, is given. This ubiquitous species occurs in the mud snail, *Nassa obsoleta* (Say).

The taxonomy and relationships of the Microphallidae are discussed.

LITERATURE CITED

Africa, C. M., and E. Y. Garcia, 1935. Heterophyid trematodes of man and dog in the Philippines with descriptions of three new species. *Philip. Jour. Sci.*, **57**: 253–267.

Brandes, G., 1888. Helminthologisches. *Arch. Naturg., Jg.*, **54**: 247–251.

Cable, R. M., and A. V. Hunninen, 1938. Observations on the life history of Spelotrema nicolli n. sp. (Trematoda: Microphallidae) with the description of a new microphallid cercaria. *Jour. Parasitol.*, **24** (Supplement): 29–30.

Carrère, P., 1936. Sur le cycle évolutif d'un *Maritrema* (Trématodes). *Comp. Rend. Acad. Sci. Paris*, **202**: 244–246.

Jägerskiöld, L. A., 1900. Levinsenia (Distomum) pygmaea Levinsen, ein genitalnapftragendes Distomum. *Centralb. Bakt.*, **27**: 732–740.

Jägerskiöld, L. A., 1901. Tocotrema expansum (Crepl.) (= Monostomum expansum Crepl.) eine genitalnapftragende Distomide. *Centralb. Bakt.*, **30**: 979–983.

Jägerskiöld, L. A., 1904. Scaphanocephalus expansus (Crepl.), eine genitalnapftragende Distomide. *Res. Swed. Zoöl. Exp. Egypt*, part **1**: 1–16.

Jägerskiöld, L. A., 1907. Zur Kenntnis der Trematoden-Gattung Levinseniella. *Zool. Stud. till. Tullberg Uppsala*, pp. 135–154.

Lebour, M. V., 1905. Notes on Northumbrian trematodes. *Northumb. Fish. Rep.*, 8 pp.

Lebour, M. V., 1907. Larval trematodes of the Northumberland Coast. *Trans. Nat. Hist. Soc. Northumb., N. S.*, **1**: 437–454, 500–501.

Lebour, M. V., 1908. Trematodes of the Northumberland Coast, No. II. *Trans. Nat. Hist. Soc. Northumb., N. S.*, **2**: 1–20.

Lebour, M. V., 1911. A review of the British marine cercariae. *Parasitol.*, **4**: 416–456.

Lebour, M. V., 1914. Some Larval trematodes from Millport. *Parasitol.*, **7**: 1–11.

M'Intosh, W. C., 1865. The trematode larva and ascaris of the Carcinus maenas. *Quart. Jour. Mic. Sci., N. S.*, **5**: 201–204.

McMullen, D. B., 1937. A discussion of the taxonomy of the family Plagiorchiidae Luhe, 1901, and related trematodes. *Jour. Parasitol.*, **23**: 244–258.

Nicoll, W. A., 1907. Observations on the trematode parasites of British birds. *Ann. Mag. Nat. Hist., Ser.* 7, **20**: 245–271.

Nicoll, W. A., 1909. Studies on the structure and classification of the digenetic trematodes. *Quart. Jour. Micros. Sci., N. S.*, **53**: 391–487.

Odhner, T., 1905. Die Trematoden des arktischen Gebietes. *Fauna Arctica*, **4**: 291–372.

Pelseneer, P., 1906. Trématodes parasites de mollusques marins. *Bull. Sci. France Belgique*, **40**: 161–186.

Rees, W. J., 1936. Note on the ubiquitous cercaria from Littorina rudis, L. obtusata and L. littorea. *Jour. Marine Biol. Ass'n.*, **20**: 621–624.

Rothschild, M., 1936. Preliminary report on the trematode parasites of Peringia ulvae (Pennant) 1777. *Nov. Zool.*, **39**: 268–269.

Rothschild, M., 1937. Notes on the excretory system of the trematode genus

Maritrema Nicoll, 1907, and the systematic position of the Microphallinae Ward, 1901. *Ann. Mag. Nat. Hist., Ser.* 10, **19**: 355–365.

Sewell, R. B. S., 1922. Cercariae Indicae. *Ind. Jour. Med. Res., Supplement,* 373 pp.

Sheldon, A. J., 1938. Studies on the life cycle of Maritrema medium (Trematoda) and a redescription of the species. *Jour. Parasitol.,* **24**: 259–262.

Sinizin, D. F., 1911. La génération parthénogénésique des trématodes et sa postérité dans les mollusques de la Mer Noire. *Mém. Acad. Imp. Sci. St. Petersbourg, Ser.* 8, **30** (5): 1–127.

Stiles, C. W., and A. Hassall, 1902. Notes on parasites 58–62. *Bull. Bur. Animal Ind.,* **35**: 19–24.

Stossich, M., 1899. Los membramento dei Brachycoelium. *Bull. Soc. Adriat. Sci. Nat. Trieste,* **19**: 7–10.

Stunkard, H. W., 1930. The life history of Cryptocotyle lingua (Creplin), with notes on the physiology of the metacercariae. *Jour. Morph. Physiol.,* **50**: 143–191.

Stunkard, H. W., 1932. Some larval trematodes from the coast in the region of Roscoff, Finistère. *Parasitol.,* **24**: 321–343.

Stunkard, H. W., 1938. Parasitic flatworms from Yucatan. *Carnegie Inst. Wash. Pub. No.* **491**: 33–50.

Tubangui, M. A., and C. M. Africa, 1938. The systematic position of some trematodes reported from the Philippines. *Philip. Jour. Sci.,* **67**: 117–127.

Villot, M. A., 1879. Organisation et développement de quelques espèces de trématodes endoparasites marins. *Ann. Sci. Nat. Zoöl., Ser.* 6, **8**: 40 pp.

Vogel, H., 1934. Der Entwicklungszyklus von Opisthorchis felineus (Riv.) nebst Bemerkungen über die Systematik und Epidemiologie. *Zoologica,* **33** (86): 103 pp.

Ward, H. B., 1901. Notes on the parasites of lake fish. III. On the structure of the copulatory organs in Microphallus nov. gen. *Trans. Amer. Micros. Soc.,* **22**: 175–187.

Witenberg, G., 1929. Studies on the trematode-family Heterophyidae. *Ann. Trop. Med. Parasitol.,* **23**: 131–239.

Yamaguti, S., 1934. Studies on the helminth fauna of Japan. Part 3. Avian trematodes, II. *Jap. Jour. Zoöl.,* **5**: 543–583.

Young, R. T., 1938. The life history of a trematode (Levinseniella cruzi ?) from the shore birds (Limosa fedoa and Catoptrophorus semipalmatus inornatus). *Biol. Bull.,* **74**: 319–329.